

Influence of Supplemental, Dietary Vitamin A on Retinol-Binding Protein Concentrations in the Plasma of Preruminant Calves^{1,2}

B. J. Nonnecke,* M. P. Roberts,† J. D. Godkin†
R. L. Horst,* D. C. Hammell,‡³ and S. T. Franklin‡⁴

*Periparturient Diseases of Cattle Research Unit,
USDA, ARS, National Animal Disease Center, Ames, IA, 50010-0070

†Department of Animal Science, University of Tennessee, Knoxville, 37901-1071

‡Dairy Science Department, South Dakota State University, Brookings, SD 57007

ABSTRACT

Transport of retinol (vitamin A alcohol) from retinoid stores in the liver to target tissues is accomplished exclusively by a specific plasma protein, retinol-binding protein. Within individuals, retinol-binding protein concentrations in plasma are regulated and remain constant except in extremes of vitamin A nutriture or in disease. In the present study, retinol-binding protein concentrations in plasma from preruminant calves supplemented with 0, 1700 (i.e., current NRC requirement), 34,000, or 68,000 IU of vitamin A daily from birth to 27 d of age ($n = 6/\text{treatment}$) were quantified. Retinol-binding protein concentrations at birth averaged 21 $\mu\text{g}/\text{ml}$ ($n = 24$) or approximately 50% of concentrations in dairy heifers and cows. Plasma retinol and retinol-binding protein concentrations were correlated positively, corroborating the role of vitamin A nutriture in the regulation of retinol-binding protein secretion from the liver. In this regard, dietary vitamin A influenced positively retinol and retinol-binding protein concentrations and, as a consequence, the degree of saturation of retinol-binding protein with retinol. At 27 d of age, calves fed $\geq 34,000$ IU of vitamin A had substantially higher retinol and retinol-binding protein concentrations than did calves fed ≤ 1700 IU of vitamin A, indicating that dietary vitamin A effects positively vitamin A status. The data also suggest that the current

NRC requirement may not be sufficient to assure vitamin A adequacy in preruminant calves. Percent saturation of retinol-binding protein with retinol in all calves was $<35\%$, much lower than anticipated and suggests that the retinol requirement of vitamin A-responsive tissues exceeded vitamin A availability.

(**Key words:** preruminant calf, vitamin A, retinol-binding protein)

Abbreviation key: RBP = retinol-binding protein.

INTRODUCTION

Delivery of retinol from the liver to vitamin A-responsive peripheral tissues is accomplished exclusively by a specific transport protein, retinol-binding protein (RBP). Retinol-binding protein is a single polypeptide of about 21,000 Da with one binding site for one molecule of all-trans-retinol. In the circulation, RBP exists in a 1:1 molar complex with another protein, transthyretin (formerly called prealbumin). Formation of the RBP-transthyretin complex is thought to reduce glomerular filtration and renal catabolism of RBP. In general, concentrations of RBP in peripheral blood are highly regulated and remain constant except in extremes of vitamin A nutriture or in disease (Smith and Goodman, 1979). In both vitamin A deficiency and in cases of vitamin A toxicity, RBP concentrations in peripheral blood decline dramatically (Smith and Goodman, 1979).

First isolated in 1968 (Kanai et al., 1968), RBP has been studied extensively in humans and rats (Soprano and Blaner, 1994). The role of RBP in the metabolism of vitamin A in dairy cattle has also received considerable attention. Bovine RBP has a molecular weight, AA composition, and fluorescence spectra that are indistinguishable from human RBP (Berni et al. 1990; Heller, 1975). Although the liver is considered the predominant source of RBP in the species studied, a variety of extrahepatic tissues appear to contain RBP mRNA, RBP protein, or both (Soprano and Blaner, 1994). Recent research indicates RBP is also produced in the bovine endometrium and that RBP expression in the bovine

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Corresponding author: B. J. Nonnecke; e-mail: bnonnecke@nadc.ars.usda.gov.

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³Present address: Merrick's Inc., P. O. Box 99, Union Center, WI, 53962.

⁴Present address: Animal Science Department, University of Kentucky, 408 W. P. Garrigus Bldg., Lexington, 40546-0215.

uterus is modulated by ovarian steroid (MacKenzie et al., 1997). These findings suggest an important role for vitamin A in bovine reproduction.

Information regarding the concentration of RBP in the peripheral blood of dairy cattle and factors influencing its concentration is limited. In a recent study (Lindberg et al., 1999), RBP concentrations in serum from periparturient dairy cows were evaluated by an enzyme immunoassay for bovine RBP. Mean RBP concentrations at 4 wk before calving ranged from 42 to 44 $\mu\text{g/ml}$ and at 8 wk after calving ranged from 55 to 78 $\mu\text{g/ml}$. Because dietary protein affected plasma RBP concentrations at 1 wk before calving, the authors suggest that the analysis of serum RBP may be useful in the assessment of AA availability in transition dairy cows. Their results also indicate that plasma concentrations of RBP in adult cows are comparable to plasma concentrations in the adult human (45 $\mu\text{g/ml}$) (Rask et al., 1980). With regard to other ruminants, serum RBP concentrations range from 36 $\mu\text{g/ml}$ in bison to 96 $\mu\text{g/ml}$ in mountain goats (Burri et al., 1993).

There is a dearth of information regarding RBP in the preruminant calf and factors affecting its concentration in the peripheral circulation of the calf. Objectives of the present study were to evaluate the effect of supplemental dietary vitamin A on the concentration of RBP in the peripheral blood of milk replacer-fed calves during the first month of life, and secondly, to examine the relationship between plasma concentrations of RBP and retinol.

MATERIALS AND METHODS

Animal Procedures

Male Holstein calves were from a commercial dairy farm located in eastern South Dakota. Immediately after birth and before being fed colostrum, calves were transported to the South Dakota State University Research and Teaching Facility where they were housed outdoors in individual calf hutches. Twenty-four calves completed the study between August 28 and December 5, 1996. Calves were assigned randomly at birth to treatments ($n = 6/\text{treatment}$) of 0, 1700, 34,000, or 68,000 IU of vitamin A daily as water dispersible retinyl acetate (Microvit A Prosol 500, Rhone Poulenc, Atlanta, GA). Supplementation with 1,700 IU/d approximated the current NRC (1989) recommendation for young large-breed calves; whereas, 34,000 IU/d approximated the amount of vitamin A provided daily by commercial milk replacers at the time of the study. The Institutional Animal Care and Use Committee of the South Dakota State University, Brookings, approved the calf-related procedures.

Table 1. Composition of low vitamin A milk replacer as provided by manufacturer (DM basis).¹

Ingredients	Analysis
CP, %	20.02
Crude fat, %	20.02
Crude fiber, %	0.15
Ash, %	8.30
Swine metabolizable energy, Kcal/kg	4019
Lactose, %	44.45
Ca, %	0.95
P, %	0.75
Na, %	0.62
Mg, %	0.14
K, %	1.77
Vitamin D ₃ , KIU/kg	10.00

¹Formulated and analyzed by Milk Specialties Inc., Dundee, IL. No vitamin A added; however, endogenous concentrations were equivalent to 506 IU vitamin A/kg of milk replacer (Hammell et al., 2000). Calves supplemented orally with 0, 1700, 34,000, or 68,000 IU of vitamin A daily. Vitamin E provided at 100 IU/d as a dietary supplement. Other vitamins that were supplemented included thiamine, riboflavin, vitamins B₆ and B₁₂, pantothenic acid, niacin, folic acid, and biotin.

Colostrum was collected, pooled, and frozen before the study was initiated. Each calf was fed colostrum at 5% of BW a single time. Colostrum was given by esophageal feeder to calves that failed to suckle. Calves were fed a custom-formulated, low vitamin A milk replacer (Milk Specialties Co., Dundee, IL) at 5% of BW supplemented with one of four vitamin A treatments 12 h after the first feeding. Diets were reconstituted to approximately 1 part milk replacer to 9 parts, warm clean water immediately before feeding. Composition of the milk replacer, provided by the manufacturer (Milk Specialties Co.), is given in Table 1. Calves were fed twice daily at approximately 0630 and 1830 h. The amount of milk replacer fed daily was adjusted on a weekly basis to 10% of BW to compensate for weight gain. Beginning at 4 d of age, calves were offered fresh water after the morning feeding. Calf starter was not fed during the study.

Calves were bled by jugular venipuncture immediately after birth (before being fed colostrum) and at 1, 6 (5.9 ± 0.3), 13 (12.7 ± 0.3), 20 (19.7 ± 0.3), and 27 d (26.7 ± 0.3) of age. Blood was collected into Vacutainers containing heparin (Becton Dickinson Vacutainers Systems, Rutherford, NJ) placed on ice, and stored in the dark until recovery of plasma. Plasma was recovered by centrifugation ($1000 \times g$, 18°C, 20 min) and was then frozen (-20°C).

Quantification of Plasma Retinol

Sample collection and processing were performed under yellow light to minimize isomerization of endogenous retinoids. Retinol in plasma was quantified by

reverse-phase HPLC by using a modification of the method originally described by Kaplan et al. (1987). All solvents, with exception of hexane, were HPLC or spectrophotometric grade (Fisher Scientific, Itasca, IL). The HPLC was performed using a Waters ALC/GPC 204 liquid chromatograph (Waters Associates, Milford, MA). Retinol was detected by using a fixed wavelength detector (Waters 440 Absorbance Detector, Waters Associates, Milford, MA) to monitor absorbance at 280 nm. External standards consisted of all-trans-retinol (0.87 to 2.62 nM) (Eastman Kodak Co., Rochester, NY). A detailed description of the method and typical chromatograms of retinoids obtained by this procedure have been published (Horst et al., 1995).

Quantification of Plasma RBP

Plasma concentrations of RBP were quantified by the method of MacKenzie et al. (1997) based on the original method of Signorella and Wymer (1984). Reagents consisted of RBP isolated and purified from fetal calf serum by DEAE-cellulose chromatography and gel filtration (MacKenzie et al., 1997), antibody to bovine RBP prepared as described previously (Liu et al., 1990), goat anti-rabbit IgG alkaline phosphatase conjugate (Bio-Rad, Hercules, CA), and phosphatase substrate (p-nitrophenyl phosphate disodium hexahydrate, Kiergaard & Perry, Gaithersburg, MD). Plates were washed with an Ultrawash II Microplate Washer (Dynatech Laboratories, Chantilly, VA), and absorbances (405 nm) were read on a Biotek Automated Microplate Reader (EL311SL, Biotek Instruments, Winooski, VT). Results were calculated using Kineticalc software (Bio-Tek).

Control serum from an adult cow was run on three independent positions of each microtiter plate. Test and control samples were diluted 1/100 in assay diluent. The final mixture in each well consisted of 15 μ l of diluted sample, 185 μ l of diluent, and 200 μ l of antibody solution. Each sample was assayed in triplicate, and time points for a particular animal were assayed on the same plate. Values for all standards were used to prepare a composite standard curve (Figure 1). The correlation coefficient for the standard curve was 0.99. Absolute values of RBP were based on comparisons with a known amount of a bovine serum albumin standard. The value of the standard was based on a Coomassie protein assay (Pierce, Rockford IL) and protein assays of this type are used as an indicator of variability between proteins. The coefficient of variation of triplicate wells was <5%, and between plates was 3.7%. Sensitivity of the assay typically was <0.01 ng/ml. Cross-reactivity to transthyretin (prealbumin), β -lactoglobulin A, and β -lactoglobulin B was undetectable.

Statistical Analysis

Data were assessed for normality prior to statistical analysis. Arithmetic or log₁₀-transformed data were analyzed as a split plot with repeated measures ANOVA using StatView software (version 5.0, SAS Institute, Inc., Cary, NC). The dietary concentrations of vitamin A and their interactions constituted the main plot, and the age of calves was the repeated measure or the split plot. Fisher's protected-LSD test was applied when treatment effects ($P \leq 0.05$) were detected by the model. Pearson's product-moment correlations were computed between retinol and RBP. Values used in these determinations represented mean plasma concentrations of retinol and RBP for calves from birth (d 0) to 27 d of age.

RESULTS

Effects of dietary vitamin A on growth performance, health, and fat-soluble vitamin status of calves in the

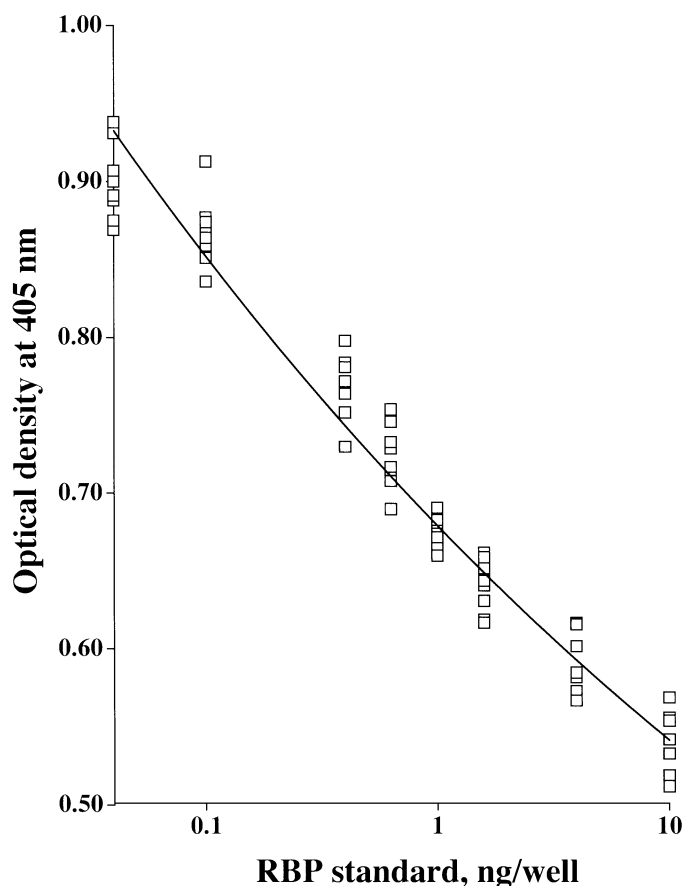


Figure 1. Standard curve used to estimate plasma retinol-binding protein (RBP) concentrations in preruminant calves supplemented with 0, 1700, 34,000, or 68,000 IU of vitamin A daily. The r^2 value for the linear/log plot was 0.9742. The equation for the line was: $Y = 0.5069 + [(0.9349 - 0.5069)/(1 + (X/0.6547)^{0.8814})]$.

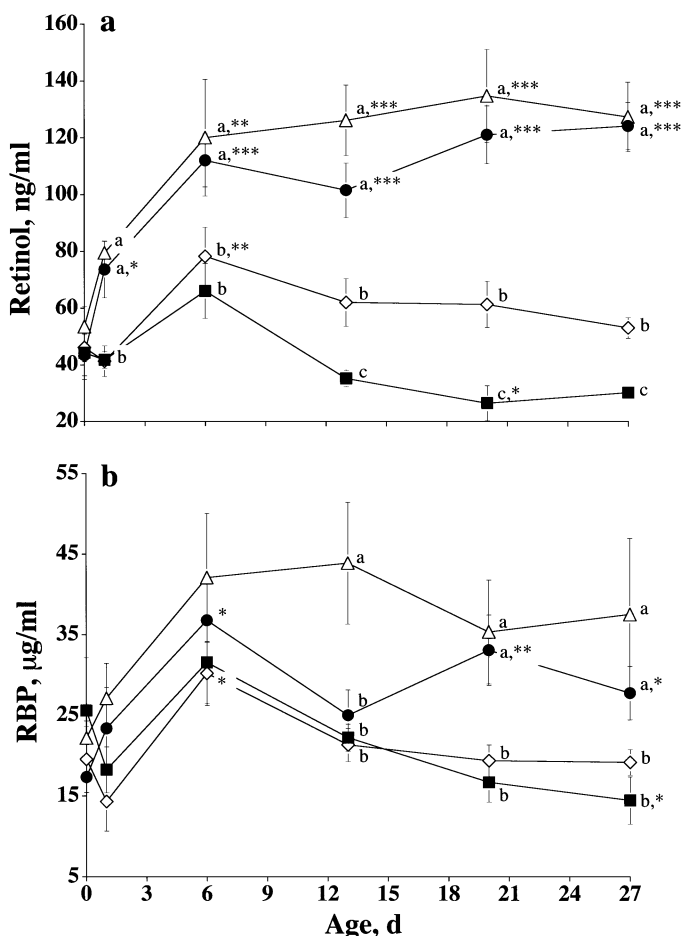


Figure 2. Mean concentrations (\pm SEM, $n = 6$ /treatment group) of retinol a) and retinol-binding protein (RBP) b) in plasma from preruminant calves supplemented with 0 (■) 1700 (◇), 34,000 (●), or 68,000 IU (Δ) IU of vitamin A daily from 1 to 27 d of age. Letters indicate treatment means that differed ($P \leq 0.05$) at a specific time. Asterisks indicate times points within a treatment that differed from d 0 value, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

present study have been reported (Ametaj et al., 2000; Hammell et al., 1998; Hammell et al., 2000; Nonnecke et al., 2000a).

Plasma Retinol Concentrations

At birth (d 0), before ingestion of colostrum and initiation of treatments, group differences in mean plasma retinol concentrations were not significant (Figure 2a). The mean retinol concentration at d 0 was 47 ng/ml. Retinol concentrations in calves supplemented with 34,000 (107 ng/ml) and 68,000 IU (118 ng/ml) of vitamin A were not different during the treatment period and they exceeded concentrations in calves given 0 (40 ng/ml) and 1700 (59 ng/ml) IU of vitamin A daily (Table 2). Least square means of the interaction of week and treatment indicated that retinol concentrations in

calves given 1700 IU of vitamin A daily (NRC requirement) were higher than retinol concentrations in unsupplemented calves from 13 to 27 d of age (Figure 2a). At 27 d of age, retinol concentrations in calves given 0, 1700, 34,000, and 68,000 IU of vitamin A averaged 31, 53, 125, and 128 ng/ml, respectively (Figure 2a).

Within each treatment group, effects of age on plasma retinol were significant (Figure 2a). Retinol concentrations in calves supplemented with $\geq 34,000$ IU of vitamin A were higher at ≥ 1 d of age than at birth (d 0). In calves given 1700 IU of vitamin A, retinol concentrations at d 0 were not different from values on d 1, 13, 20, and 27, but were lower than d 7 values. In unsupplemented calves, retinol concentrations declined progressively from d 7 to 27 and by d 20 were lower than at birth.

Plasma RBP Concentrations

Group differences in mean plasma RBP concentrations were not significant at birth (Figure 2b). The mean RBP concentration at d 0 was 21.2 μ g/ml. Mean RBP concentrations in calves supplemented with 0 (21 μ g/ml) and 1700 (20.9 μ g/ml) IU of vitamin A were not different at any time during the treatment period (Table 2 and Figure 2b) even though calves fed 1700 IU of vitamin A daily had higher retinol concentrations than unsupplemented calves at 13, 20, and 27 d of age (Figure 2a). Effects of supplemental vitamin A on retinol concentrations were evident at 1 d of age (Figure 2a); however, treatment effects on RBP were not evident until 13 d of age (Figure 2b). Least squares means of the interaction of week and treatment indicated that RBP concentrations were significantly higher in calves given 68,000 (44 μ g/ml) IU of vitamin A than in calves given 0 (22 μ g/ml), 1700 (21 μ g/ml), and 34,000 (25 μ g/ml) IU of vitamin A at 13 d of age (Figure 2b). At 20 d, RBP concentrations in calves supplemented with 34,000 (33 μ g/ml) and 68,000 (35 μ g/ml) IU of vitamin A exceeded those in calves given 0 (17 μ g/ml) and 1700 (19 μ g/ml) IU of vitamin A daily. At 27 d, RBP concentrations in calves supplemented with $\geq 34,000$ IU of vitamin A were higher than in calves given ≤ 1700 IU of vitamin A daily. Mean RBP concentrations in 27-d-old calves fed 0, 1700, 34,000, and 68,000 IU of vitamin A daily were 15, 19, 27, and 38 μ g/ml, respectively.

Age-dependent changes in RBP were significant in calves given 0, 1700, and 34,000 IU of vitamin A daily, but not in calves supplemented with 68,000 IU of vitamin A (Figure 2b). Plasma RBP concentrations in calves supplemented with 1700 and 34,000 IU of vitamin A exceeded d 0 values on one or more occasions during the treatment period. Unsupplemented calves were the only group in which RBP concentrations during the

Table 2. Effect of supplemental dietary vitamin A on plasma concentrations of retinol and retinol-binding protein (RBP) in milk replacer-fed, preruminant calves.¹

Variable	Vitamin A, IU/d							
	0		1,700		34,000		68,000	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Retinol (ng/ml)	40.1 ^c	3.5	59.3 ^b	3.8	106.7 ^a	5.2	117.8 ^a	8.3
RBP (μg/ml)	20.7 ^b	1.7	20.9 ^b	1.5	29.2 ^a	2.0	37.2 ^a	3.6
RBP (% saturation)	16.5 ^c	1.8	22.9 ^b	1.8	28.4 ^a	1.8	25.6 ^{ab}	1.8

^{a,b,c}Means within a row with no common superscript differ ($P \leq 0.05$).

¹Mean retinol and RBP concentrations during the treatment period (1 to 27 d of age).

treatment period were lower than at birth (15 μg/ml at d 27 vs. 26 μg/ml at d 0).

Relationships between Retinol and RBP

The correlation between plasma concentrations of retinol and RBP in all calves 0 to 27 d of age was positive ($r = 0.70$, $P < 0.0001$) (Figure 3). Associations between retinol and RBP from birth to 27 d postpartum were also examined within each treatment-group. Correla-

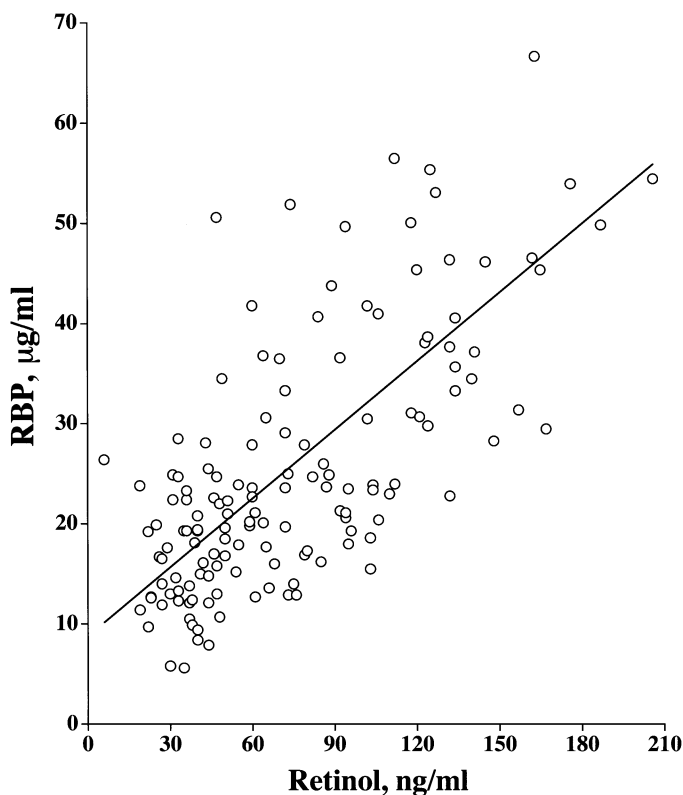


Figure 3. Relationship between retinol and retinol-binding protein concentrations in plasma from milk replacer-fed calves from birth to 27 d of age. Points ($n = 144$) on graph correspond to values for 24 calves sampled on 6 separate occasions. The positive linear relationship defined by: $y = 0.229x + 8.796$, $r = 0.702$, $P < 0.0001$.

tion coefficients between retinol and RBP for 0, 1700, 34,000, and 68,000 IU vitamin A groups were 0.58 ($P < 0.001$), 0.56 ($P < 0.001$), 0.66 ($P < 0.0001$), and 0.71 ($P < 0.0001$), respectively.

Because one mole of RBP has one binding site for one mole of retinol and retinol is transported exclusively by RBP in the peripheral circulation (Rask et al., 1980), the molar ratio of retinol to RBP ($\times 100$) provided an estimate of the percent saturation of RBP with retinol. Effects of dietary treatments on percent saturation of RBP are presented in Figure 4 and Table 2. At birth (d 0), group differences in RBP saturation were not significant. The mean saturation of RBP at d 0 was 25%. During the treatment period, RBP saturation was higher in calves supplemented with 1700 (25%), 34,000 (31%), and 68,000 (29%) IU of vitamin A than in calves not supplemented with vitamin A (18%) (Table 2). Least square means of the interaction of day and treatment indicated treatment-specific differences in RBP saturation were significant at 6, 13, 21, and 27 d of age (Figure 4). From 13 and 20 d of age, RBP saturation was higher in calves supplemented with 1700, 34,000, and 68,000 IU of vitamin A than in unsupplemented calves. Saturation of RBP in 27-d-old calves given 0, 1700, 34,000, and 68,000 IU of vitamin A was 23, 24, 38, and 32%, respectively. At this age, saturation in calves given 34,000 IU of vitamin A daily exceeded that in calves given 0 and 1700 IU of vitamin A.

The effect of age on RBP saturation was not significant in calves supplemented with 0, 1700, and 68,000 IU of vitamin A daily but was significant in calves given 34,000 IU of vitamin A (Figure 4).

DISCUSSION

Results from the present study indicate that the concentration of RBP in plasma from the preruminant milk replacer-fed calf is influenced directly by the amount of vitamin A in the diet. There were pronounced treatment-specific differences in plasma concentrations of RBP from 13 to 27 d of age, with mean RBP concentrations in calves supplemented with $\geq 34,000$ IU of vita-

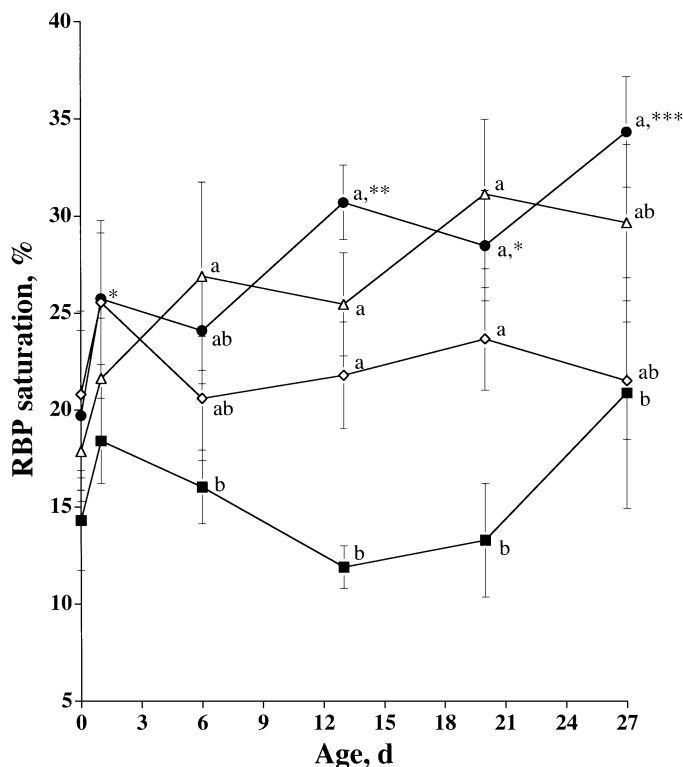


Figure 4. Mean percent saturation (\pm SEM, $n = 6/\text{treatment}$) of plasma retinol-binding protein (RBP) with retinol in preruminant calves supplemented with 0 (■), 1700 (◇), 34,000 (△) and 68,000 (●) IU of vitamin A daily from 1 to 27 d of age. The degree of saturation of RBP by retinol was calculated using molecular weights of retinol (286.5 Daltons) and RBP (21,000 Daltons). Letters indicate treatment means that differed ($P \leq 0.05$) at a specific time. Asterisks indicate times points within a treatment that differed from d 0 value, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

min A exceeding concentrations in calves supplemented with ≤ 1700 IU of vitamin A during the experimental period. This observation is in agreement with studies in other species indicating that dietary vitamin A specifically regulates the amount RBP released by the liver (Soprano and Blaner, 1994).

Plasma RBP was detectable in all calves shortly after birth (d 0), suggesting RBP is present in the liver at birth and is available to transport vitamin A from the liver to peripheral tissues. The mean plasma concentration of RBP at birth (i.e., $21.2 \mu\text{g/ml}$), however, was substantially lower than plasma concentrations reported for dairy cows ($\geq 42 \mu\text{g/ml}$) (Lindberg et al., 1999) and other adult ruminants (Burri et al., 1993). The mean concentration of RBP ($37.6 \mu\text{g/ml}$) in 27-d-old calves supplemented with 68,000 IU of vitamin A approached RBP concentrations in the plasma of cows ($\geq 40 \mu\text{g/ml}$) (Lindberg et al., 1999), whereas the value ($19.2 \mu\text{g/ml}$) for calves fed the NRC (1989) requirement (i.e., 1700 IU of vitamin A daily) was substantially

lower. Vitamin A deficiency and the associated reduction in the availability of retinol specifically blocks secretion of RBP from the liver (Smith and Goodman, 1979). As a consequence, plasma RBP concentrations decrease with time. Because the mean plasma RBP concentration in unsupplemented calves was significantly lower at 27 d of age ($14.5 \mu\text{g/ml}$) than at birth ($25.6 \mu\text{g/ml}$), calves in this group likely were deficient with regard to vitamin A. These data suggest that the vitamin A provided by colostrum (a single feeding at 5% of BW) and the low vitamin A milk replacer was not sufficient to maintain plasma RBP and retinol concentrations.

Although plasma retinol concentrations also were impacted positively by increased dietary vitamin A, retinol concentrations in all calves during the treatment period were substantially lower than those considered suggestive of a vitamin A deficiency (i.e., $<200 \mu\text{g/ml}$) in growing Holstein calves (Eaton et al., 1970). In calves given 0 and 1700 IU of vitamin A daily, mean retinol concentrations were substantially lower than retinol concentration (i.e., $100 \mu\text{g/ml}$) considered indicative of more advanced deficiency (Eaton et al., 1970). Because dietary vitamin A and hepatic reserves of vitamin A regulate plasma RBP concentrations (Soprano and Blaner, 1994), it is not surprising that calves supplemented with 0 and 1700 IU of vitamin A daily had substantially lower plasma RBP and retinol concentrations than calves fed 34,000 and 68,000 IU of vitamin A daily. These results suggest that supplementing the diet of the preruminant, milk replacer-fed calf with the current NRC requirement (i.e., 1700 IU/d) for vitamin A does not assure vitamin A adequacy.

Concerns exist regarding the potential impact of relatively high levels of vitamin A present in commercial milk replacers on the growth and health of preruminant calves. In studies of acute vitamin A toxicity in rats (Mallia et al., 1975), circulating RBP concentrations decrease dramatically, and most of the vitamin A present in the plasma is as retinyl esters in association with lipoproteins. In the present study, supplemental vitamin A as high as 34,000 and 68,000 IU daily did not impact negatively plasma RBP concentrations in preruminant calves, an indication that supplementation at 20 to 40 times the NRC recommendation did not affect negatively vitamin A homeostasis. Retinyl ester concentrations in plasma were not determined in the present study. A recent study (Nonnecke et al., 2000b) found that plasma concentrations of retinyl esters remain low ($<1 \text{ ng/ml}$) in calves supplemented with ≤ 4000 IU of vitamin A daily and become elevated (22 ng/ml) in calves supplemented with 34,000 IU of vitamin A daily, suggesting that providing vitamin A at 34,000 IU daily exceeds the needs of the preruminant calf.

Because each RBP molecule has one binding site for one molecule of retinol and essentially all retinol in the circulation is transported in association with RBP (Smith and Goodman, 1979; Soprano and Blaner, 1994; Underwood, 1994), the percent saturation of RBP with plasma concentrations of RBP and retinol can be estimated. The low saturation of RBP with retinol in the preruminant calf during the first month of life, regardless of dietary treatment, was surprising given data from studies evaluating plasma RBP and retinol concentrations in adult cattle and other species. With plasma RBP and retinol concentrations from a recent study of vitamin A transport in dairy cattle (Lindberg et al., 1999), the estimated percent saturation of RBP with retinol is substantially higher in dairy cows (i.e., 92.4%) and heifers (i.e., 69.8%) than in calves (13.0 to 37.5%) in the present study. In a study evaluating plasma retinol and RBP concentrations in women and their full-term infants, RBP saturation was found to be approximately 100%, even though retinol and RBP concentrations were substantially lower in infants than in mothers (Basu et al., 1994). Causes of the low saturation of RBP with retinol in the calf are not known; however, it is possible that the retinol requirement of vitamin A responsive tissues exceeds the capacity of the liver to produce and release holo-RBP (retinol-RBP-transthyretin complex) into the circulation. Additional research is necessary to determine effects of dietary vitamin A on the concentrations of retinoids and their carrier proteins in vitamin A responsive tissues as well as liver of neonatal calves.

Plasma RBP has been suggested as a surrogate for plasma retinol in assessing vitamin A status (Underwood, 1994) because RBP is the sole mechanism of the transport of retinol from liver to tissues (Soprano and Blaner, 1994). The ELISA-based assay for RBP (MacKenzie et al., 1997) also is less technically demanding than the HPLC assay for retinol (Kaplan et al., 1987), a benefit in population studies examining vitamin A status of dairy cattle. Results from the present study demonstrated a positive correlation between circulating retinol and RBP concentrations in preruminant calves, indicating that plasma RBP concentrations may be useful in estimating the vitamin A status of newborn calves. Because circulating RBP concentrations are influenced by moderate to severe malnutrition and acute infection (Soprano and Blaner, 1994), an RBP assay may have limited value in estimating vitamin A status of malnourished or diseased calves.

In conclusion, the concentration of RBP in plasma from preruminant calves was substantially lower than concentrations in the circulation of adult dairy cattle. Concurrent low RBP and retinol concentrations in the plasma of the preruminant calf may be a reflection of

age rather than true deficiency. Providing vitamin A in excess of the current NRC recommendation markedly improved the vitamin A status (i.e., plasma retinol concentrations) of preruminant, milk replacer-fed calves and may benefit the growth and health of the calf.

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